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14. ABSTRACT

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In this project, characteristics of biogenic structures in the sediment including number, diameter, and depth are correlated with benthic communities dwelling in hypoxic, intermittently hypoxic, and normoxic conditions using non-destructive Computed Tomography (CT) imagery of sediment cores and Sediment Profile Imaging (SPI) photography. Biogenic structures are also correlated with sediment physical properties, bioturbation rates, and bioturbation behaviors (dilator or compactor) of benthic invertebrate fauna. Initial data for this project was collected during two cruises along the continental shelf of Louisiana, the first in April 2009 and the

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The Impact of Hypoxia on Bioturbation Rates in the Louisiana Continental Shelf, Northern Gulf of Mexico

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***Abstract*—Variation in the spatial and temporal extent of hypoxia in coastal bottom waters of the northern Gulf of Mexico leads to changes in benthic community structure and sediment physical properties. Past and present benthic community structure determines what types of biogenic structures are present in the sediment as well as faunal mixing rates. Therefore, hypoxia has an important effect upon bioturbation. This study focuses upon the effects of hypoxia on bioturbation specifically on the continental shelf of Louisiana, where hypoxia has become an important issue due to its seasonal reoccurrence and increasing expansion across the northern Gulf of Mexico over the past 30 years.**

In this project, characteristics of biogenic structures in the sediment including number, diameter, and depth are correlated with benthic communities dwelling in hypoxic, intermittently hypoxic, and normoxic conditions using non-destructive Computed Tomography (CT) imagery of sediment cores and Sediment Profile Imaging (SPI) photography. Biogenic structures are also correlated with sediment physical properties, bioturbation rates, and bioturbation behaviors (dilator or compactor) of benthic invertebrate fauna. Initial data for this project was collected during two cruises along the continental shelf of Louisiana, the first in April 2009 and the second in September 2009. Four different "provinces" were chosen for sampling using bottom water oxygen concentration data from the Louisiana Universities Marine Consortium (LUMCON) and archived sediment type data. These four sampling sites represent normoxic and hypoxic provinces with a consistent sediment type. The provinces consist of a normoxic site (NO) that has experienced hypoxia less than 25% of the time and briefly hypoxic (BH), frequently hypoxic (FH) and hypoxic (HO) sites that have experienced hypoxia greater than 25% of the time.

We expect areas of rapid deposition (organics and inorganic sediment) and low bottom water oxygen to be colonized by a few organisms that are concentrated near the sediment-water interface, remaining above the depth of the redox potential discontinuity (RPD). In contrast, well oxygenated areas are expected to have developed diverse benthic communities that have attained the equilibrium stage of succession, which includes larger, deeper burrowing fauna. The diverse, deeper-burrowing benthos helps to create and maintain a deeper RPD. As a consequence of the vertical zonation and faunal diversity differences between stressed and recovered areas, several sediment properties may be affected.

SPI photographs show that RPD depths in the four provinces vary from 2 to 6 cm below the sediment surface in the spring, before hypoxia has developed. The x-radiographs depict the extent of recent sedimentation and bioturbation at each province. Featured in the x-radiographs are: event layers, shell fragments, voids above and below event layers, vertical, horizontal, and complex burrows. Preliminary radiochemistry results for the April 2009 cruise indicate shallow depths of sediment mixing at all sites on a temporal scale of ~1 year (Be-7), though provinces HO and FH show a deeper mixing depth (2.25 cm) over this period than provinces BH or NO (1.25 and 1.75 cm, respectively). This may reflect a competitive advantage of discrete macrobenthic organisms who are better able to tolerate hypoxic conditions, a rapid re-colonization of previously hypoxic bottom sediments, or both. Results from the April 2009 cruise are presented as baseline data to establish benthic macrofauna densities and sediment properties before development of hypoxia. A second cruise was conducted in September to assess the effects of seasonal hypoxia at these same four provinces.

I. Introduction

Hypoxia is present in bottom waters around the world and in recent years it has become more prevalent, especially in coastal areas such as the northern Gulf of Mexico. In fact, previous studies indicated that hypoxia is a reoccurring seasonal phenomenon on the Louisiana continental shelf, first appearing in the spring, intensifying in the summer, and declining in the fall [1]. When bottom waters become hypoxic (<2 mg/l of O_2), macrofauna living in the water column and in upper layers of the sediment may be adversely affected and die if bottom water oxygen concentrations drop too low [2]. The underlying cause of hypoxia in this region is a matter of ongoing debate, but the conventional explanation states that hypoxia on the northern Gulf of Mexico shelf is a result of high nutrient and organic matter inputs, oxygen consumption due to organic decomposition (*i.e.*, eutrophication) and water column density stratification [3]. Fertilizers released in the Mississippi River watershed enrich riverine inputs with nitrogen and phosphorus, and the nutrient-rich freshwater inputs overlie the dense, highly saline Gulf waters [4]. Today the northern Gulf of Mexico is the second largest zone of coastal hypoxia in the world.

The spatial and temporal extents of hypoxia on the continental shelf of Louisiana vary from year to year. Hypoxia from the Mississippi River bird-foot delta and across the Louisiana shelf to Texas was first reported in 1985 and each year since. The total

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area of hypoxia has increased from ~8,000 km^2 in 1985 to, most recently, an estimated 22,000 km^2 [5]. Due to spatial and temporal variability, regions are exposed to hypoxia for discrete time periods. An exposure gradient develops that may affect benthic communities and sediment properties differentially, according to hypoxic condition severity or duration. Although changes in benthic communities in relation to hypoxic stress have been documented, the effects of these changes on sediment properties have not been studied.

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According to a model developed by Pearson and Rosenberg [6], there is a well-defined pattern related to faunal species, abundance, and biomass that occurs along a gradient of increasing organic matter in the benthic marine environment. According to this model, as the sediment becomes more enriched with organic matter, the sediment becomes increasingly eutrophic and the environment shifts from a "normal" state with a redox potential discontinuity (RPD) depth deep in the sediment, to a "transitory" state with a shallower RPD, and finally to a "polluted," anaerobic state with RPD at the sediment surface. "Normal" zones of organic matter enrichment are characterized by high macrofaunal diversity, large-sized macrofauna, deep-burrowing macrofauna, and high sediment porosity. "Polluted" zones with very high organic matter enrichment are characterized by low macrofaunal diversity, small-sized macrofauna, non-burrowing macrofauna, and low sediment porosity. "Transitory" zones are characterized by properties lying somewhere along the gradient from "normal" to "polluted." In this study, we expect to find some semblance of this pattern described by Pearson and Rosenberg apparent in our sediment cores that were obtained from provinces with bottom water ranging from normoxic to hypoxic. Thus, it is hypothesized that benthic communities with low organism abundance, small organisms and low biodiversity will be found in areas with low oxygen (hypoxic provinces), and that the infauna will be confined to the uppermost sediment layers. In contrast, communities with high organism abundance, larger organisms and high biodiversity are expected in areas with well oxygenated bottom waters (normoxic province). Provinces having fauna with characteristics somewhere in between the two extremes are expected in areas defined by frequency and recent history of hypoxia.

Hypoxia directly affects the benthic community and therefore indirectly affects benthic biological activities. It is important to study the mixing rate of benthic macrofauna and their associated bioturbation structures for a number of reasons. When macrofauna move, ingest, egest, build structures, and perform various other activities in the sediment, they affect the microtopography of the surrounding environment. This result, in turn, has various effects upon the overlying water column, influencing the environment in various ways. For example, turbulence and transport of materials at the sediment-water interface are subject to interference by biogenic structures present in surface sediment layers [7],[8]. Other properties affected by bioturbation include but are not limited to the following: sediment chemistry and diagenesis [9], larval and juvenile recruitment of various biota [10], mechanical properties of the sediment-water interface such as hardness and surface cohesion [11], and acoustic signal absorption and reflection [12].

II. MATERIALS AND METHODS

Initial data for this project was collected during two cruises along the continental shelf of Louisiana, the first in April 2009 and the second in September 2009. Four different "provinces" were chosen for sampling using bottom water oxygen concentration data from the Louisiana Universities Marine Consortium (LUMCON) and archived sediment data. These four sampling sites represent normoxic and hypoxic provinces with a consistent sediment type. The provinces consist of a normoxic province (NO) that has experienced hypoxia less than 25% of the time, and briefly hypoxic (BH), frequently hypoxic (FH), and hypoxic provinces (HO) that have experienced hypoxia greater than 25% of the time. The BH, FH, and HO provinces differed in terms of their individual frequencies and recent histories, of seasonal hypoxia.

At each province a total of six 50-cm×50-cm box cores were retrieved from either two or three stations. From each box core seven or eight subcores were collected including three subcores for identification and counting of invertebrate infauna (biocores), three subcores for measurement of physical, chemical, and acoustic properties (PCA cores), one subcore for measuring permeability, and one x-radiograph subcore. Methodology for processing varied depending upon the type of subcore:

A) Biocores

Biocores were collected from the box core using 8.2-cm-inside-diameter polycarbonate tubes measuring approximately 30 cm in length. The biocores were sectioned into 10 sample intervals: the first two sections at 1-cm intervals (0-1 and 1-2 cm), the next four sections at 2-cm intervals (2-4, 4-6, 6-8, and 8-10 cm), and up to four sections at 5-cm intervals (10-15, 15-20, 20-25, and 25-30 cm). The biocores were manually extruded with a piston-like device pushing from the bottom of the core. Extruded intervals were measured, and sediment was cut with a thin wire and removed with a spatula. The slices were sieved through a 300-µm nitex screen attached to the bottom of a 15-cm-diameter, 30-cm-long PVC pipe. Samples were rinsed in a 285-l conical-bottom vat using flowing seawater to carry the fine sediment overboard. After the sample was sieved, the screen was removed from the pipe and placed in a labeled container filled with a 5% formalin-seawater solution by volume, rose Bengal vital stain, and sodium borate buffer. These samples were transported back to the laboratory for preservation in 70% isopropanol, sorting, and identification of fauna with a dissecting microscope.

B) Physical, Chemical, and Acoustic Property Subcores:

Three PCA subcores were collected from each box core using 5.9-cm-inside-diameter polycarbonate tubes approximately 48 cm in length. Sediment sound speed and attenuation were logged aboard ship for each of the 72 subcores [13]. Twelve PCA cores were transported back to the laboratory for organic matter and radionuclide analyses and five PCA cores were killed with formalin for imaging with Computed Tomography (CT).

Radionuclide analyses of Pb-210, Be-7, unsupported Th-234, and Cs-137 were performed in order to determine sediment accumulation rates and bioturbation depths and mixing rates. The radionuclides Th-234, ($t_{1/2}$ = 24 d) and Be-7, ($t_{1/2}$ = 53 d) are useful for evaluating rapid mixing processes that occur on the order of weeks to months, whereas Cs-137 and Pb-210 are appropriate for quantifying relatively long-term mixing on the order of years to decades. Lead-210 activity concentrations were determined according to methodology described by [14-19] and modified by [20]. Sediment samples ($\approx$1 g) were spiked with the tracer Po-209 and completely digested with HF, HCl, and HNO_3 on a hot plate. After this a silver disc was added to each sample and heated in order to provide a substrate for the spontaneous deposition of polonium. Alpha spectrometry was employed to resolve Pb-210 profiles for sediment sampled from subcores collected from all four provinces. Both Be-7 and Cs-137 were determined on bulk dry sediment samples from each of the four provinces by non-destructive gamma spectrometry.

C) X-radiograph Subcores:

Two 43×33×3 (L×W×Thickness) cm "slab" subcores were collected for each of the four provinces. With one of the four sides of the subcore box removed, the subcore was inserted into the sediment in the box cores. The missing side was then inserted into guides on the box allowing the sediment to be enclosed within the subcore box, excavated from the box cor,e and capped. Elastic cords were used to secure the bottom and the subcore was removed from the box core and cleaned of any adhering sediment. The subcores were x-rayed at 50 kV and 20 mA.

Sediment Profile Imaging (SPI) photographs were also taken during the April 2009 cruise at each of the four provinces using a SPI camera remotely deployed from the ship. This instrument operates by penetrating the sediment with a slowly descending wedge-shaped chamber with a clear port on the vertical face, which contains a digital camera that is hard-wired back to the ship SPI photography is a fast and efficient technique that has been used since the early 1970s in order to document organism-sediment relationships [21]. SPI photography can be used to monitor both physical-chemical parameters (*e.g.*, grain size, sediment surface relief, redox area, redox contrast, and relict redox boundaries) and biological parameters (*e.g.*, number and taxa of epifauna and infauna, depth and density of tubes, tube types, number and depth of feeding voids, apparent species richness, and successional stage) [22].The SPI camera captures images of the sediment-water interface and provides information about the types of macrofauna present in the sediment, what types of burrows are created by bioturbating macrofauna, evidence of storms, and approximate location of the RPD where surface oxidized sediment is separated from the reduced sediment below. Hundreds of SPI photographs were obtained during the April 2009 cruise so that the best quality images could be selected to provide information about each type of province; that is, normoxic (NO), briefly hypoxic (BH), frequently hypoxic (FH), and hypoxic (HO).

III. Results and Discussion

Initial data for this study were collected during the April 2009 cruise along the continental shelf of Louisiana. These data comprise the baseline against which data from the September 2009 cruise will be compared to determine how the seasonal reoccurrence of hypoxia affects biogenic structures in the sediment and macrofaunal mixing depths and rates. Results from the September 2009 cruise will be discussed in later publications.

According to the Pearson-Rosenberg model [6], "normal" benthic communities with low organic matter input tend to live in a sedimentary environment with a deep RPD that can reach depths of over 10 cm, whereas organic-matter-enriched benthic communities tend to live in sediments with a shallow RPD less than 3 cm deep. "Normoxic" benthic communities should be dominated by infaunal deposit-feeders such as "head-down" conveyor-belt feeders, tubicolous polychaetes, and mobile and free-living species. The effects of these species on the sediment, which include the transfer of particles over vertical distances up to 20 cm and intensive particle mixing, should result in homogenized sediments, void spaces produced by feeding, and a rough sediment-water interface covered with feeding pits and fecal or excavation mounds [22].

We see a relatively rough, undulating seafloor surface in SPI photographs from the NO province in accordance with the equilibrium stage stated in the Pearson-Rosenberg model (Fig. 1). The RPD is recognizable by the transition from oxygenated, light brown sediment at the surface to a dark gray, reduced layer lying underneath. The RPD appears to be nominally at 2 cm below the interface, with indications of excursions down to 5 and 6 cm depth. Some of the brownish oxidized mud deeper than 2 cm is a result of "draw down" from the top layer of sediment during penetration of the SPI wedge, but the area on the right of the image is almost certainly in situ oxidized sediment. A worm in its burrow is seen near the center of the image at 4 cm depth. Another SPI photograph from the NO province illustrates an RPD with a feeding void at 2 cm depth and evidence of a burrow with a sediment slurry effluent plume (Fig. 2). The sediment-water interface in Fig. 2 appears to have at least one feeding pit. A deep dwelling (5 to

9 cm sediment depth) worm and an RPD extending down to 3 to 6 cm is apparent in Fig. 3. The presence of carbonate shell hash throughout the sediment is apparent in all three images from this province (Figs. 1-3).

An x-radiograph from the NO province depicts the large amount of carbonate shell material present in the sediment at this site (Fig. 4). The shells are apparent as light-colored inclusions within a darker matrix of sediment (lighter color indicates greater density to x-rays in this x-radiograph "positive"). The random orientation of the shell pieces is indicative of a lack of coherent bedded deposits and is illustrative of biogenic mixing. The darker layer at the sediment surface is the low-density (high-porosity) surface layer of the sediment. This layer is nominally 2 cm in thickness, but has occasional thicker sections (Fig. 4). A few dark areas among the lighter colored sediment matrix indicates mottling of the sediment fabric, which is likely an indication of biogenic mixing down to 12 cm sediment depth. Burrows are not well defined in x-radiograph images from NO province. The large concentration of shell material (up to 22% by weight [13]) scatters much of the x-rays and the lower density structure is lost. Some fine laminae indicative of a storm layer are apparent at the horizon between the darker, low-density surface later and the lighter, denser sediment below [13].

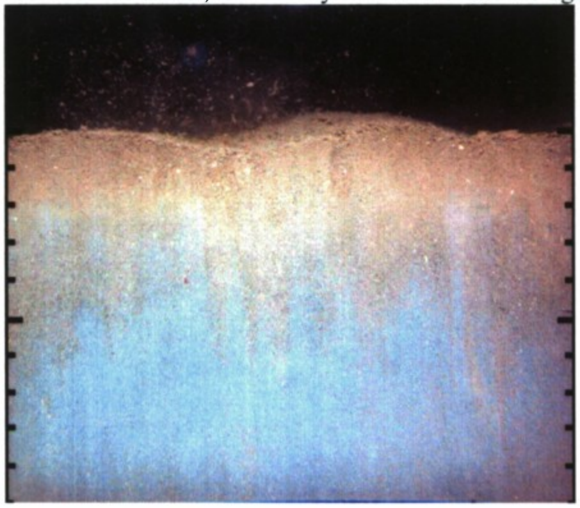

Figure 1. SPI photograph from the NO province showing a rough interface and oxidized sediment down to 6 cm depth. Graduated tics on sides are 1 cm.

Figure 2. SPI photograph from the NO province showing evidence of burrow and feeding void. Graduated tics on sides are 1 cm.

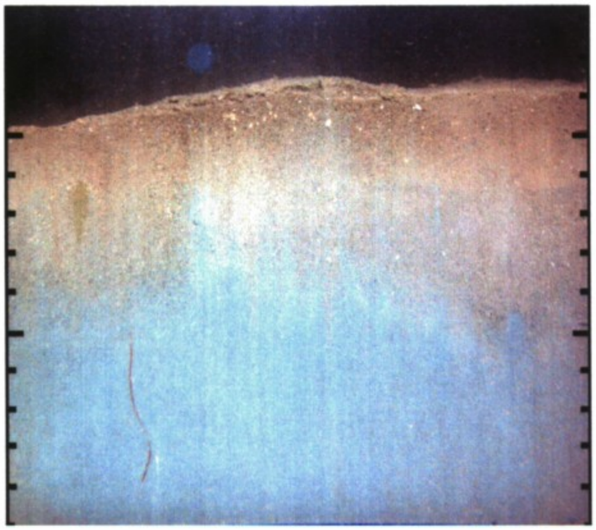

Figure 3. SPI photograph from the NO province showing evidence of a worm and 3-5 cm deep RPD. Graduated tics on sides are 1 cm.

Figure 4. X-radiograph of a subcore from the NO province.

In contrast, "polluted" or "pioneering" benthic communities are dominated by small, opportunistic, tube-dwelling polychaetes or oligochaetes that tend to form dense aggregations. The effects of pioneering species are limited to the upper 3 cm or less of the sediment and include the effects of tube structures on sedimentation and erosion, lack of sediment rigidity, particle bioturbation, and fecal pellet accumulation on the top of the sediment resulting from surface deposit and suspension feeding [22]. The depth of the RPD in a hypoxic province should be reduced from that found in sediment from a normoxic province.

The SPI photograph from the hypoxic province (HO) reveals small tubes of benthic infauna protruding from the sediment surface (Fig. 5). The depth of the RPD is 3 to 5 cm, although there are isolated zones of oxidized sediment amid the reduced sediments below the RPD. The sediment below the RPD is darker than the reduced sediments at the normoxic province. It is not known if these reduced sediments are higher in sulfides (or have a lower reduction potential value) than those at the NO province, but the amount and quality (in terms of the C:N ratio) of the organic matter, which usually drives the oxidation-reduction reactions in the sediment, at this province is about the same as at the NO province [13].

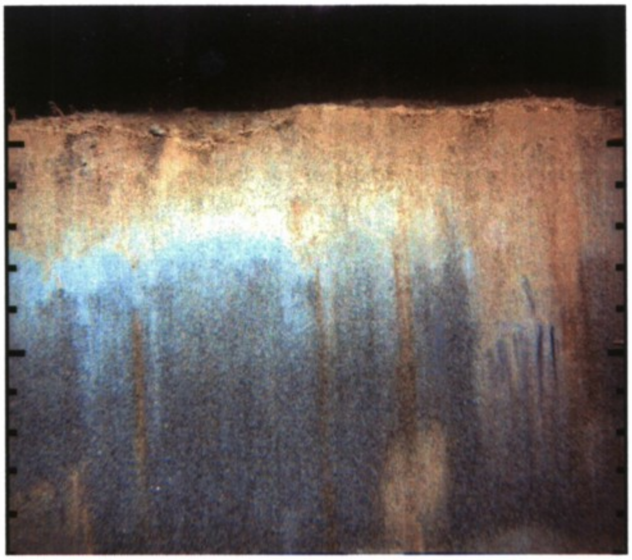

Figure 5. SPI photograph collected at province HO showing numerous small tubes protruding from the sediment-water interface. Graduated tics on sides are 1 cm

An x-radiograph from the HO province reveals the large number of biogenic structures within the sediment at this site (Fig. 6). Loose, low-density sediment (~3 cm thick) on the surface overlays a thin, higher-density laminated deposit (likely storm layer). There are a large number of burrows (those lined with compacted sediment stand out as lighter colored cylinders of greater density in this x-radiograph "positive"). Burrows are both vertical (narrow bore, center) and U-shaped (large bore, left and right edges). A large, reworked area of low density comes into view from left to right at about 17 to 21 cm depth. Most of the U-shaped burrows contain higher-density sediment, which could be coarser grains from storm sedimentation that infiltrated abandoned burrows, creating "death assemblages" [23]. Because many of the burrows are light-colored, indicating higher density sediment, most of the biogenic structures at the HO province could be relict—persisting from a biological community that predated the relatively recent hypoxic conditions in the sediments.

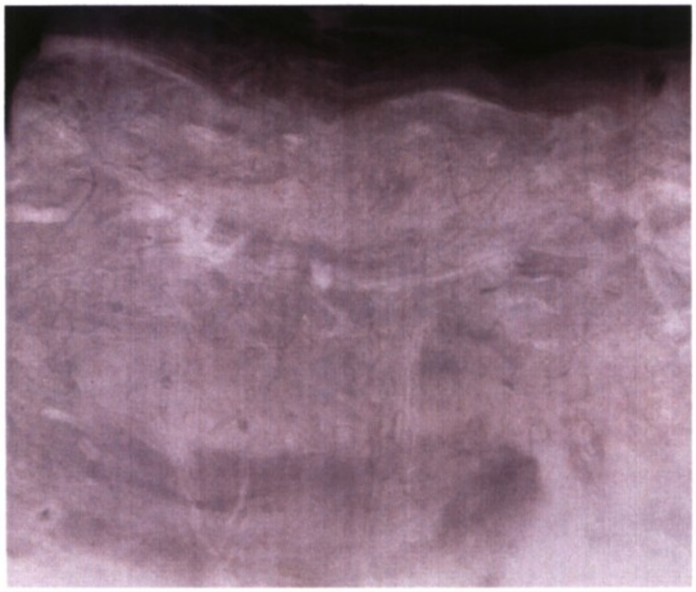

Figure 6. X-radiograph of a subcore from the HO province showing numerous and various burrows. The sediment depth at the bottom of the image is 21 cm.

SPI photographs from the other hypoxic provinces (BH and FH) show sedimentary structures similar to those at HO province (Figs. 7 and 8). Both of these images show a granular surface sediment texture to the surface sediment, which may be fecal pellets from surface deposit and suspension feeders. The SPI photograph from BH province (Fig. 7) shows tubicolous infauna (left and center, top) and possible surface suspension feeders (right, top). The SPI photograph from FH province shows a fluffy, rough sediment-interface

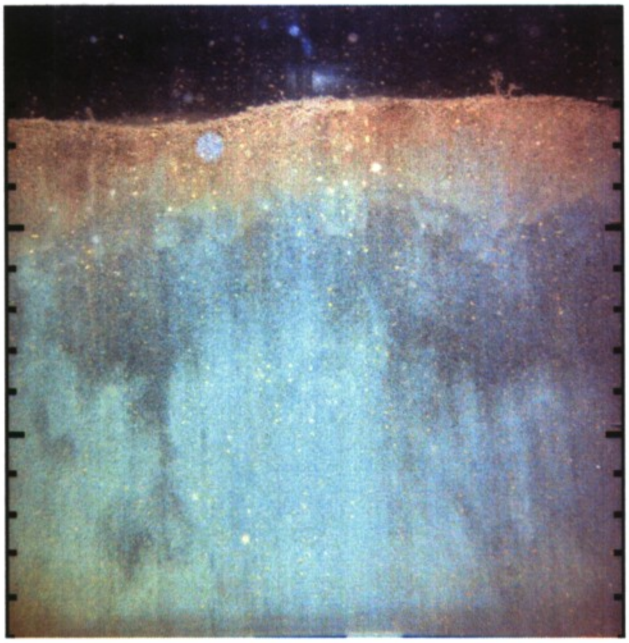

Figure 7. SPI photograph collected from the BH province showing infaunal tubes at the sediment-water interface. Graduations on sides are 1 cm.

Figure 8. SPI photograph collected at the FH province showing shallow and deep burrows. Graduations on sides are 1 cm.

possibly due to biogenic activity. Inhabited, active burrows at the surface and at 13 to 17 cm sediment depth are also visible in the photograph (Fig. 8). In this image, the RPD is highly variable, contracting to near the interface and expanding down to 8 cm depth in the sediment.

For sediment cores collected in April 2009, preliminary radiochemistry data indicates shallow depths of sediment mixing at all sites on a temporal scale of ~1 year (Be-7), though sediments from the most frequently and persistently hypoxic provinces (HO and FH) show a deeper mixing depth (2.25 cm) over this period than the rarely hypoxic (BH) or normoxic (NO) sites (1.25 and 1.75 cm, respectively). This may reflect a competitive advantage of discrete macrobenthic organisms that are better able to tolerate hypoxic conditions [24-25], a rapid re-colonization of previously hypoxic bottom sediments [26-27], or both. While radionuclides are generally unable to distinguish between mixing derived from physical versus biological processes, the short temporal resolution of Be-7 and lack of major storms on the Louisiana shelf in the year prior to sampling suggests that these data reflect biological mixing.

IV. Summary

SPI photographs from the April 2009 cruise are useful in defining the type of environment apparent in the benthos before the occurrence of hypoxia. The redox potential discontinuity (RPD) layer is a distinct feature in most of the SPI photographs taken during the April 2009 cruise. Photographs that clearly show the RPD layer indicate that the RPD lies between 2 and 6 cm below the sediment surface in all provinces examined. The RPD is recognizable by the transition from oxidized, light-brown sediment at the surface to a dark gray, reduced layer lying underneath. The RPD layer is indicative of the type of benthic community present in the sediment because the deeper the RPD layer, the deeper oxygen is penetrating into the sediment. Other important features visible in SPI photographs include burrowing worms, feeding voids, biogenic interface roughness, and tubicolous infauna.

The x-radiographs provide visual images used to depict the extent of recent sedimentation and bioturbation at each province. X-radiographs from the April cruise show biogenic structures not readily imaged in the single plane obtained with the SPI camera. Although no direct evidence of the RPD can be seen in x-radiographs, the lower-density, bioturbated sediment contrasts well within a higher-density, more consolidated sediment matrix. Moreover, high-density features such as shell material, silt- and sand-sized sediments in storm layers are readily apparent in the x-radiographs.

Photographs and x-radiographs from the four provinces indicate the following: the normoxic (NO) province sediment has biogenic interface roughness, is well mixed, and has a variable RPD due to deep-burrowing infauna; the hypoxic (HO) province sediment has numerous tubicolous infauna at the sediment-water interface and a diverse assortment of burrows below the RPD, which may include abandoned relict burrows; the briefly hypoxic (BH) province sediment has tubicolous infauna at the surface, granular, uncompacted surface sediment, and possible surface deposit and suspension feeders; and the frequently hypoxic (FH) province sediment has a fluffy, uncompacted, rough interface and active burrows down to at least 17 cm depth. Macrofauna analysis from the four provinces would appear to agree with the photographic and x-radiographic evidence. The sectioned, sieved, and sorted samples indicate that BH and NO provinces have the highest abundance of fauna, with HO and FH provinces having much less numerous fauna [28]. These biological data suggest that BH and NO provinces also have the most diverse fauna, with FH province having the least diverse fauna.

CT-imagery and complete results from the radionuclide analyses were not available from the cruises at the time of publication. For sediment cores collected in April 2009, preliminary radiochemistry data indicates shallow depths of sediment mixing at all sites on a temporal scale of ~1 year (Be-7), though sediments from the most frequently and persistently hypoxic provinces (HO and FH) show a deeper mixing depth (2.25 cm) over this period than the rarely hypoxic (BH) or normoxic (NO) sites (1.25 and 1.75 cm, respectively). Comparison of these results with new data collected in September 2009 will be published in future contributions.

Acknowledgment

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